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Impact of heavy metals and biochemical parameters on specific leaf area of roadside trees in Kathmandu, Nepal

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ABSTRACT

Kathmandu is seriously facing the air pollution problem because of the rapid increase in vehicular emissions. Most commonly grown roadside trees like *Populus euroamericana, Ficus religiosa, Jacaranda mimosifolia, Grevillea robusta, Callistemon lanceolatus* and *Cinnamomum camphora* from different sites of Kathmandu were collected during the winter season to measure specific leaf area (SLA), biochemical parameters and heavy metals (Cu, Pb and Zn) content. The relations among them (SLA, biochemical parameters, heavy metals) were also studied. Specific leaf area (SLA) ranged from 24.48± 0.87cm^2 g-1 to 188.08±35 cm^2 g-1 and the highest SLA was observed in *Jacaranda mimosifolia* (188.08±35cm^2 g-1). The mean value of biochemical parameters such as total chlorophyll (TCh) ranged from 0.64mg/g in *Cinnamomum camphora* to 1.243 mg/g in *Ficus reliogiosa* whereas pH ranged from 5.28 in *Callistemon lanceolatus* to 6.75 in *Grevillea robusta*. Similarly Ascorbic acid rangedfrom 4.54 mg/g in *Cinnamomum camphora* to 8.61mg/g in *Populus euroamericana* and relative water content (RWC) from 40.02 % in *Jacaranda mimosifolia* to 86 % in *Cinnamomum camphora*. Mean value of Zn accumulation ranged from 14.83 mg/kg in *Ficus religiosa* to 71.92 mg/kg in *Populus euroamericana*, Cu accumulation from 4.14 mg/kg in *Populous euroamericana* to 14.56 mg/kg in *Jacaranda mimosifolia* and Pb accumulation from 0.05 mg/kg in *Populus euroamericana* to 22.47 mg/kg in *Callistemon lanceolatus*. Pb and Cu accumulation at phytotoxic concentrations in leaves showed negative impact on SLA.

Key words : *Tree leaves, Total chlorophyll, Ascorbic acid, Relative water content, pH, Heavy metals*

Introduction

Carbon dioxide is the most important greenhouse gas which is now 50% higher than before the industrial revolution. Increase in CO_2 is mainly due to the burning of fossil fuels (WMO, 2021). Leaves play an important role to sequester carbon dioxide through photosynthesis from the atmosphere. Leaf surface area is one of the key factors which determines the potential of photosynthesis in leaves. Specific leaf area (SLA) is a ratio of leaf area per unit leaf dry mass which describes the distribution of leaf biomass in relation to leaf area (Pierce *et al.*, 1994). Specific leaf area is highly correlated with light level and decreases with increasing light intensity (Kellomäki and Oker-Blom, 1981; Evans and Poorter, 2001), hence SLA is related to a leaf's position in the crown. Trees generally have a lower SLA at the top of the canopy because they receive direct solar radiation whereas at the lower parts of the crown may be shaded and have a higher SLA. (Nagel and O'Hara, 2001; Goudie *et al.*, 2016). SLA has been considered as one of the most important characteristics of leaf which is used to measure plant growth (Wright *et al.*, 2004**;** Lambers and Poorter, 1992; Falster *et al.*, 2018). SLA is an important indicator of plant strategies for adaptation (Grime, 2006; Westoby *et al.*, 2002) and used widely in plant ecology, agronomy, and forestry (Poorter *et al.*, 2009). The level of influence on growth depends on plant species, pollutant type and concentration along with number of environmental factors (Wuytack *et al.*, 2011; Chaturvedi *et al.*, 2013). Kapoor *et al.*, (2013) investigated tree species *Dalbergia sissoo* and found reduction in photosynthetic pigments, pH and RWC in the polluted environment. Certain heavy metals like copper and zinc are essential micronutrients for plants because they are involved in numerous metabolic processes as constituents of enzymes and other proteins. However, toxic levels of heavy metals cause several toxic symptoms in plants such as the inhibition of photosynthesis and enzyme activity, growth retardation, disturbed mineral nutrition, water imbalance and the alteration of membrane permeability (Sharma and Dubey, 2005; Pandey *et al.*, 2009).

Kathmandu, the capital of Nepal is highly affected by air pollution due to the increase number of automobiles, densely populated and its unique bowl-shaped topography (Dhamala *et al.*, 2018) which prevents particulate matter freely escaping in to the atmosphere and it makes vulnerable situation in Kathmandu. Air quality of Nepal was ranked at 145th position out of 180 countries in Environmental Performance Index in 2020 (Shrestha *et al.*, 2021 and Wendling *et al.*, 2020), and Kathmandu is listed as one of the most polluted cities in the world (Subedi, 2021). Most of the previous studies related with plants and air pollution were mainly focused in Air Pollution tolerance Index (APTI) and heavy metal accumulation. But it is not cleared if the toxic effect of heavy metal are reflected on SLA or not. It is also not clear if SLA is dependent on different physiological parameters like RWC, pH, total chlorophyll and ascorbic acid. Therefore, the relation of heavy metal accumulation and the biochemical parameters (like total chlorophyll, ascorbic acid, pH and relative water content) with specific leaf area have been investigated in the present study. The result of this study will identify suitable roadside trees that can accumulate heavy metals and help to mitigate air pollution problems.

Materials and Methods

The study area

Kathmandu is a valley and located at 27°42′0′′ N and 85°18′0′′ E. It ranges from 1200 to 1400 m above sea level in subtropical zone. Bowl shaped topography makes it more prone to air pollution (Pradhan *et al.*, 2012; Shrestha, 2001) and characterized by a typical monsoon climate with rainy summer and dry winter. An average summer temperature lies between 19 °C-35 °C and winter temperature between 2 °C-12 °C with an annual mean temperature 24 °C and an average rainfall of 1343 mm.

Study Method

Sample collection

Leaves of most commonly grown trees along the road side like *Populus euroamericana, Ficus religiosa, Jacaranda mimosifolia, Grevillea robusta, Callistemon lanceolatus* and *Cinnamomum camphora* were collected from lower surface (above 3 m from the ground). The polluted sampling sites were Tinkune, Airport, Chabhil, Samakhusi, Basundhara, Dhumbarahi, Lainchaur, Swayambhu and Balaju, and a comparatively clean site Narayanthan was considered as a control site (Fig. 1). The leaf samples were collected in winter season (December-January) and used for the measurements of specific leaf area (SLA), heavy metals and biochemical parameters (total chlorophyll, pH, ascorbic acid relative water content).

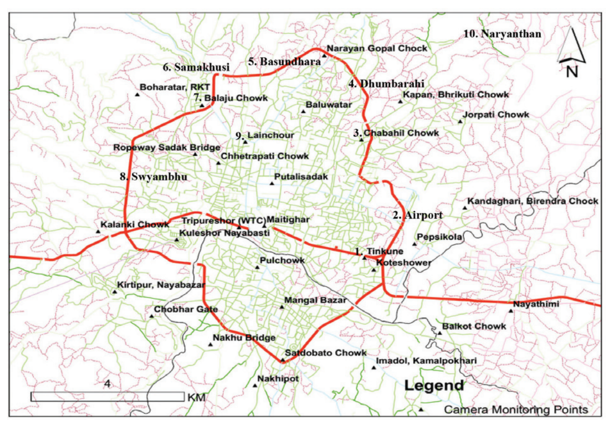

Fig. 1. Study site 1-Tinkune 2-Airport 3- Chabhil 4-Dhumbarahi 5-Basundhara 6-Samakhusi, 7-Balaju 8- Swayambhu 9-Lainchaur 10-Narayanthan

Metal Analysis

For metal analysis, the leaves were washed with running tap water; rinsed with double deionized water and then dried at room temperature (20 °C) about 24-48 hours. Again dried at 60 °C for 48 hours. The dried leaf samples were grinded using mortar and pestle to prepare representative samples. 1g DW of the representative sample was dipped in 8 ml concentrated HNO_3 (Merck). These were left over night at room temperature. On the next day, the mixture was warmed for 2 h at 50 °C and subsequently heated at 160 °C for 4 h. The cooled extracts were filtered through Ash less filter paper (What man 589) and were diluted to prepare 25 ml with double deionized water (Sawidis *et al.*, 1995c). The filtrates were then analyzed for Cu, Pb and Zn by using Perkin Elmer (2380) Atomic Absorption Spectrometer (AAS) at wavelength 324.7 nm for Cu, 283.3nm for Pb and 213.9 nm for Zn (Welz, 1985). Two plant materials of National Bureau of Standard (USA) each with Nos. 1537 (Tomato leaves) and 1575 (Pine needles) were analyzed following the same procedure and the metal recoveries obtained were 95.5%, 94.2% and 97.5% for Cu, Pb and Zn respectively. The relative standard deviation of the measurements was 2.7% for Cu. 7.5% for Pb and 3.9%).

Biochemical parameters

Total Chlorophyll

Total Chlorophyll was obtained according to Barnes *et al*., 1992). 0.05 g of leaves were cut in to smaller pieces and placed in test tubes containing 5 ml DMSO. Then test tubes were incubated in a water bath at 60-65 ºC for an hour. From preliminary studies this time was judged satisfactory for the full decolonization of tissues and kept for cooling at room temperature about 30 min. Further these were filtered and their absorptions were measured at 665 nm and 648 nm being the final stages. Measurment of absorption of blank DMSO was carried out with the help of Spectrophotometer. Finally total chlorophyll (TCh) concentration (a, b and total) was expressed as mg/g fresh weight and determined by the following formulae (Barnes *et al.*, 1992).

Chlorophyll a (mg/g F.W) = (14.85 A665 -5 .14 A648) (1)

Chlorophyll b (mg/g F.W) = (25.48 A665 – 7.36 A648) (2)

Total chlorophyll (mg/g F.W) = (7.49 A665 + 20.34 A648) (3)

Where: A665 = absorption value at 665 nm

A648= absorption value at 648 nm

Leaf extract pH

5 g of a leaf sample was crushed with the help of motor and pestle. 50 ml deionized water was added in to it and then obtained suspension was measured with a digital pH meter (Apriyantono *et al.*, 1989)

Ascorbic acid (AA)

Ascorbic acid content (expressed in mg/g) was measured according Bajaj and Kaur (1981) method.1g of the fresh leaf was taken and then 4 ml oxalic acid EDTA extracting solution was added to it. Again one ml of Orthophospheric acid and 1 ml 5% tetraoxosulphate acid was added to this mixture, and finally 2 ml of ammonium molybdate and 3 ml of water added. The solution thus obtained allowed to stand for 15 min after which the absorbance at 760 nm is measured with a spectrophotometer. The concentrations of ascorbic acid in the sample then extrapolated from a standard ascorbic acid curve.

Estimation of relative water content (RWC)

RWC was determined according to Barrs and Weatherly (1962). RWC is a ratio of the amount of water in the leaf tissue at sampling to that present when fully turgid. A composite sample of leaf discs was taken and the fresh weight was taken and then leaf was floated on water for up to 24 h. The turgid weight was then recorded, and the leaf tissue was subsequently oven-dried to a constant weight at about 85°C for 24 h and reweighted. RWC is calculated by using the following formula:

Relative water content (%) = {(F-D) / (T-D)} ×100

F = Fresh weight of leaves (g)

D = Dry weight of leaves (g)

T = Turgid weight of leaves (g)

Specific leaf area (SLA)

Seven matured leaves from lower surface of different directions around the canopy of each tree species were collected. Leaf samples were rinsed with distilled water and then photos were taken along with scales. The collected leaf samples were then dried in hot air oven at 60 °C for 24 hours. Weigh of the completely dried leaves were recorded using three digital electronic balance. The area of the photographed leaves were measured by using Image J program

(Abràmoff *et al.*, 2004). Finally, SLA (cm^2 g^{-1}) of each leaf was calculated by dividing leaf area by corresponding leaf dry weight.

Statistical Analysis

All the data analysis was conducted using IBM SPSS statistics program version 25. To evaluate the significant differences in SLA between polluted and control sites one sample t-test was conducted. Pearson correlation was calculated between the % decreased in SLA and biochemical parameters, and also between the % decrease in SLA and metal content in leaves.

Results

Metal accumulation

Mean concentrations of Zinc (Zn) was found to be higher at polluted sites than at control sites in all studied plants. Highest Zn was recorded in *Populus euroamericana* (71.92 mg/kg) and lowest in *Ficus religiosa* (14.83 mg/kg) at different polluted sites of Kathmandu (Fig. 2). Highest mean value for Zn accumulation (71.92 mg/kg) was recorded in *Populus euroamericana* and it ranged from 14.46 mg/kg to 130.57 mg/kg at different polluted sites. Lowest mean value for Zn accumulation was recorded in *Ficus religiosa* (14.83 mg/kg) and it ranged from 8.13 to 20.87 mg/kg at different polluted sites in Kathmandu.

Mean concentrations of copper (Cu) was mostly higher at control sites than at polluted sites in all studied plants. Highest Cu was recorded in *Jacaranda mimosifolia* (14.56 mg/kg) and lowest in *Populus euroamericana* (4.14 mg/Kg)) at polluted sites (Fig.3). Highest mean value (14.56 mg/kg) for Cu accumulation was recorded in *Jacaranda mimosifolia* and it ranged from 5.72 mg/kg to 20.60 mg/kg at different polluted sites. Lowest mean value for Cu accumulation was recorded in *Populus euroamericana* (4.14 mg/kg) and it ranged from 0.05 mg/kg to 11.93 mg/kg at different polluted sites in Kathmandu.

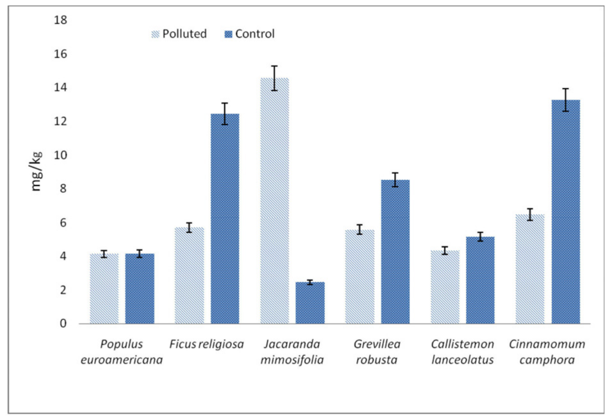

Fig. 3. Copper concentrations (mean value) in the leaves of different tree species collected from various polluted and control sites

Mean concentrations of lead (Pb) was mostly higher at polluted sites than at control sites in all studied plants. Highest Pb was recorded in *Callistemon lanceolatus* (22.47 mg/kg) and lowest in *Populus euroamericana* (0.05 mg/kg) at polluted sites (Fig.4). Highest mean value (22.47 mg/kg) for Pb

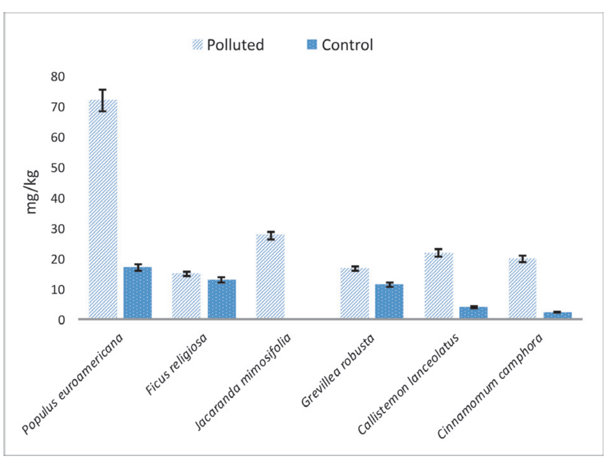

Fig. 2. Zinc concentrations (mean value) in the leaves of different tree species collected from various polluted and control sites

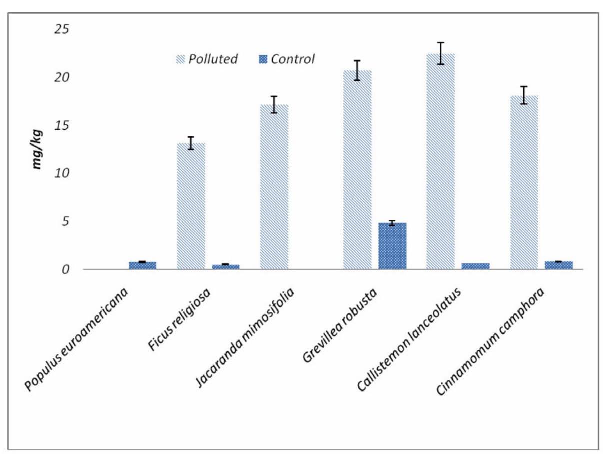

Fig. 4. Lead concentrations (mean value) in the leaves of different tree species collected from various polluted and control sites

was recorded in *Callistemon lanceolatus* and it ranged from 5.21 to 25.20 mg/kg at different polluted sites in Kathmandu.

Biochemical parameters

Mean value of total chlorophyll (TCh) was found to be higher at control sites than at polluted sites in all studied plants except in *Jacaranda mimosifolia*. Highest TCh mean value at control site was recorded in *Ficus reliogiosa* (1.243 mg/g) and lowest recorded in *Cinnamomum camphora* (0.64 mg/g). But at polluted sites highest TCh mean value was recorded in *Jacaranda mimosifolia* and lowest in *Cinnamoum camphora* (Fig. 5). Mean value of pH was mostly found to be higher at control sites than at polluted sites in all studied plants except in *Populus euroamericana* and *Jacaranda mimosifolia*. Highest pH mean value at control site was recorded in *Grevillea robusta* (6.75) and lowest recorded in *Jacaranda mimosifolia* (5.43). But at polluted sites highest pH mean value was recorded in *Ficus religiosa* (6.33) and lowest in *Callistemon lanceolatus* (5.28)(Fig. 6). Mean value of Ascorbic acid(AA) was generally found to be higher at control sites than at polluted sites in all studied plants except in *Grevillea robusta and Cinnamomum camphora*. Highest Ascorbic acid mean value at control site was recorded in *Populus euroamericana* (8.61mg/g) and lowest recorded in *Cinnamomum camphora* (4.54 mg/g). But at polluted sites highest Ascorbic acid mean value was recorded in *Populus euroamericana* (8.10 mg/g) and lowest in *Ficus religiosa* (4.80 mg/g) (Fig 7). Mean value of Relative water content (RWC) was generally found to be

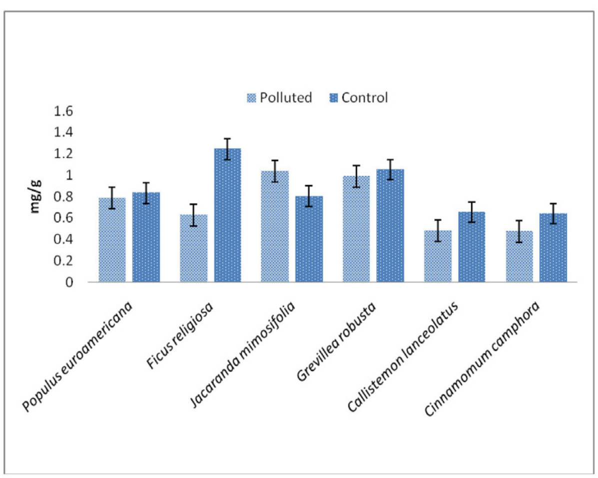

Fig. 5. Total chlorophyll content (mean value) in the leaves of different tree species collected from various polluted and control sites

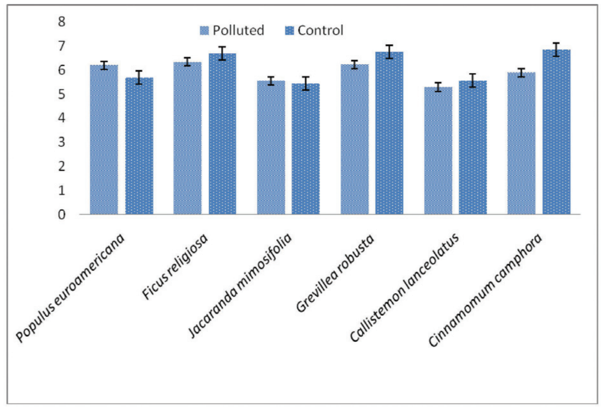

Fig. 6. pH (mean value) in the leaves of different tree species collected from various polluted and control sites

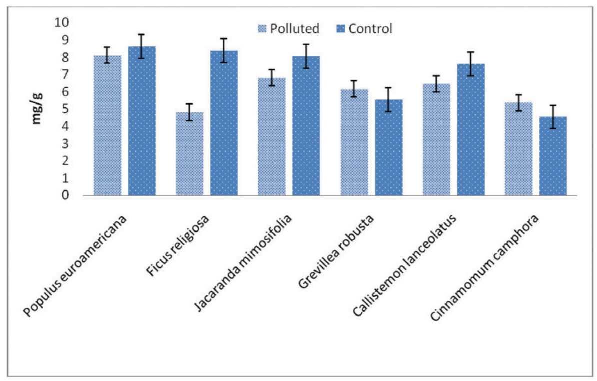

Fig. 7. Ascorbic acid (mean value) in the leaves of different tree species collected from various polluted and control sites

higher at control sites than at polluted sites in all studied plants except in *Ficus religiosa* and *Jacaranda mimosifolia*. Highest Relative water content (RWC) mean value was recorded in *Cinnamomum camphora* (86 %) and lowest recorded in *Jacaranda mimosifolia* (40.02 %) at control site. But at polluted sites highest Relative water content (RWC) mean value was recorded in *Ficus religiosa* (80.78 %) and lowest in *Grevillea robusta* (60.51%) (Fig. 8).

Specific leaf area (SLA)

Specific leaf area (SLA) in all the studied tree leaves decreased at polluted sites than at control sites. Specific leaf area (SLA) of different species and the SLA percentage decreased at polluted sites are given in Table 1. In this study SLA among the studied tree leaves ranged from 188.08±35.12 to 24.48± 0.87$cm^2 g^{-1}$. Decrease in SLA in polluted sites ranged from 78.18± 4.47 to 9.32±4.80 %. Though all the tree spe-

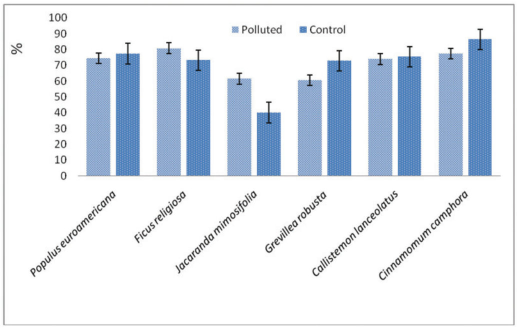

Fig. 8. Relative water content (meanvalue) in the leaves of different tree species collected from various polluted and control sites

cies showed decrease in SLA at polluted sites but was significant (at P=0.05) in *Populus euroamericana* (at Tinkune, Airport, Dhumbarahi), *Ficus religiosa* (at Dhumbarahi, Basundhara, Balaju, Airport), *Jacaranda mimosifolia* (at Lainchaur, Basundhara, Swayambhu), *Grevillea robusta* (at Lainchaur, Basundhara) and in *Callistemon lanceolatus* (at Dhumbarahi, Balaju, Airport, Swayambhu).

Correlation between SLA and Bio chemicals

Results of Pearson correlations between SLA and biochemical parameters like total chlorophyll (TCh), pH, ascorbic acid (AA) and relative water content

Table 1. SLA ($cm^2 g^{-1}$) and percentage decreased in SLA of different tree species growing along the road side in various polluted (P) and control sites (C) in Kathmandu.

Plants	Experimental (P) and control (C) site	SLA Mean ± Sd	%Decreased in SLA Mean ± Sd	t-value*
Populus euroamericana	Tinkune (P)	8.48±19.66	38.99±13.47	5.015*
	Airport(P)	112.73±20.60	20.69±7.68	4.668*
	Chabhil(P)	93.50±31.32	27.93±16.78	2.883
	Samakhusi (P)	89.92±37.40	34.80±21.99	2.740
	Basundhara (P)	114.90±34.87	9.32±4.80	3.363
	Dhumbarahi (P)	61.03±12.33	50.67±10.11	8.685*
	Narayanthan (C)	131.07±32.21		
Ficus religiosa	Dhumbarahi (P)	99.13±17.38	25.21±2.90	15.069**
	Basundhara (P)	81.85±6.35	34.02±14.07	4.189*
	Balaju (P)	69.31±9.00	47.31±1.79	45.790**
	Airport (P)	61.12±6.81	53.87±7.59	12.289**
	Narayanthan (C)	132.70±23.55		
Jacaranda mimosifolia	Lainchaur (P)	164.08±36.56	13.00±5.66	3.974*
	Dhumbarahi (P)	112.47±86.85	40.94±44.98	1.576
	Basundhara (P)	74.22±17.06	41.83±14.73	4.917*
	Airport (P)	128.52±4.74	23.77±14.07	2.925
	Swayambhu(P)	41.77±8.67	73.57±1.26	101.335**
	Narayanthan (C)	188.08±35.12		
Grevillea robusta	Lainchaur (P)	84.12±17.64	25.69±10.48	4.245*
	Dhumbarahi(P)	72.12±6.03	27.48±13.71	3.471
	Basundhara(P)	101.28±6.94	13.38±1.70	13.638**
	Airport(P)	80.08±25.83	28.98±19.05	2.634
	Balaju(P)	61.55±31.94	48.20±26.83	3.111
	Narayanthan(C)	114.58±29.75		
Callistemon lanceolatus	Dhumbarahi(P)	24.48± 0.87	78.18± 4.47	48.605**
	Balaju(P)	35.60±13.45	31.73±7.47	4.584*
	Airport(P)	61.79±22.33	40.84±30.55	4.793*
	Swayambhu(P)	69.31±5.66	21.05±9.11	21.211**
	Narayanthan(C)	116.15±28.74		
Cinnamomum camphora	Dhumbarahi(P)	91.11±14.18	10.90±10.83	1.743
	Basundhara(P)	88.74±9.58	32.72±22.63	2.504
	Balaju(P)	99.74±0.08	16.90±7.52	3.891
	Airport(P)	104.54±25.05	32.94±22.68	2.514
	Narayanthan(C)	139.44±37.04		

*P=0.05 and ** P=0.001 obtained from one sample t-test of % decreased SLA for each tree species with N value ranging from 15 to 21.

(RWC) is given in Table 2. Among all studied plants, % decrease in SLA of *Ficus religiosa* showed positive significance correlation with total chlorophyll (TCh) and pH. In *Jacaranda mimosifolia* and *Callistemon lanceolatus* positive significance relation was observed between % decrease in SLA and Ascorbic acid.

Correlation between SLA and metal content

Results of Pearson correlations between % decrease in SLA and heavy metal contents (Zn, Cu, Pb) in leaves is given in Table 3. Percentage decrease in SLA of *Populus euroamericana* showed significant (P=0.05) negative correlation with Zn but significant (P=0.05) positive with Pb. In *Ficus religiosa* % decrease in SLA showed positive significance correlation with Cu and Pb but in *Jacaranda mimosifolia*% decrease in SLA showed negative correlation with Zn and Cu. In *Grevillea robusta*% decrease in SLA showed negative significance correlation with Pb and in *Callistemon lanceolatus* it showed negative significance correlation with Zn. In *Cinnamomum camphora*, % decrease in SLA showed negative significane correlation with Zn but positive with Cu.

Discussion

Metal accumulation

Zn is one of the essential element in many biochemical process such as auxin, chlorophyll, carbohydrate and protein synthesis (Broadley, 2007). Onderand Dursun (2006) reported that the highest Zn concentrations were found in Cedar needles at an industrial site. Zinc accumulation in *Populus* differed from 26.1 to 139 mg kg^{-1}, in unpolluted and polluted sites in Greece (Sawidis *et al.*, 1995). In this study it was found that plants grown in urban localities accumulated more Zn than other metals. The range of Zn concentration between 10 to 150 mg kg^{-1} (Padmavathiamma and Li, 2007; Hu *et al.*, 2014) is conventional for plants and toxic concentrations range from 300 to 400 mg kg^{-1} depending on plant species (Broadley *et al.*, 2007). In the present study the highest Zn accumulations was detected in *Populus*. The amount of Zn accumulation in leaves are not at a toxic level. Copper is an essential trace element for plants. Both of its deficiency and excess affect many enzymes which catalyze oxidation and reduction reactions (Ouzounidou, 1994; Celik *et al.*, 2005; Doganlar and Atmaca, 2011). The conventional range of Cu concentrations in the plants is 3 to 30 mg kg^{-1} (Kabata and Pendias, 2001) but its phytotoxic concentrations range is 20 to100 mg kg^{-1}(Padmavathiamma and Li, 2007). In the present study, the highest increase in Cu content was found in *Jacaranda mimosifolia.* Though the mean value (14.15 mg/kg) of Cu content in it is within the nor-

Table 2. Correlations of percentage decreased in SLA with TCh, pH, AA and R WC of leaf at polluted sites

Plants	% decrease SLA-TCh	% decrease SLA-pH	% decrease SLA-AA	% decrease SLA-RWC
Populus euroamericana	0.021	-0.107	-0.217	-0.101
Ficus religiosa	0.807**	0.572*	0.356	0.0376
Jacaranda mimosifolia	0.381	0.312	0.801**	0.078
Grevillea robusta	0.169	0.227	0.212	0.266
Callistemon lanceolatus	0.175	-0.220	0.694**	-0.100
Cinnamomum camphora	0.274	0.362	-0.134	0.154

* Significant at P= 0.05 level and ** at P = 0.01 level

Table 3. Correlations of percentage decreased SLA with leaf metal content (Zn, Cu, Pb)

Plants	% decrease SLA-Zn	% decrease SLA-Cu	% decrease SLA-Pb
Populus euroamericana	-0.460*	0.091	0.737**
Ficus religiosa	-0.316	0.535*	0.599*
Jacaranda mimosifolia	-0.656**	-0.767**	0.117234
Grevillea robusta	-0.38	0.198	0.669**
Callistemon lanceolatus	-0.721**	0.285	-0.507
Cinnamomum camphora	-0.769**	0.618*	-0.35314

*Correlation is significant at P= 0.05 and **Correlation is significant at P = 0.01 level.

mal range but at some sites its concentrations were at phytotoxic range i.e. above 20 mg kg^{-1}. The sufficient or normal Pb concentration in the plants can be in the range of 5 to10 mg kg^{-1} (Kabata-Pendias and Pendias, 2001) and its toxic concentrations is from 30 to 300 mg kg^{-1}. While Markert (1994) reported the toxic Pb range for plants to be between 3 and 20 mg kg^{-1}. In this study, Pb amounts in all plants and sampling points were within normal limits and were found higher than the control site.

Biochemical parameters

In all studied tree species, biochemical parameters showed different variations in polluted and control sites. Total chlorophyll were reduced in polluted sites which were in agreement with the observations of Prusty *et al.*, 2005), and Sharma and Tripathi, 2009). The reduction in pigment concentration might be due to deposition of dust particles on leaf surfaces which reduced the light available for photosynthesis and resulted in the inhibition of chlorophyll biosynthesis (Prusty *et al.*, 2005), and the other one was due to dissolution of metals and polycyclic hydrocarbons cell sap as a result blocked the stomatal pores from exchange of air, thus developing the stress on plant metabolism and resulting in chlorophyll degradation (Kapoor *et al.*, 2013). pH is a biochemical parameter that serves as a sensitivity indicator of air pollution (Joshi *et al.*, 2011), and plants with a pH of around 7 are more pollution-tolerant. The leaf extract pH of plant species collected from polluted sites was lower than that of control sites which agreed with Rai and Panda, 2013). The decrease in leaf extract pH could be due to the effect of atmospheric SO_2 and NO_X. Ascorbic acid in plant leaves plays multiple functions to perform through cell wall synthesis, cell division, photosynthetic carbon fixation and also acts as a strong reducer protecting the plants against reactive oxygen species (ROS), thereby improving the tolerance ability of the trees against air pollution. With the increase in ascorbic acid content (Lima *et al.*, 2000), the tolerance level in plants also increases The present study revealed that most of tree species at control sites had higher ascorbic acid content than at polluted site but *Cinnamomum* and *Grevillea* had more average Ascorbic acid content at polluted sites. This indicates that the trees like *Cinnamomum* and *Grevillea* have developed better tolerance ability than others. The average value of RWC at polluted sites were slightly decreased in *Grevillea* and *Cinnanomum*, but was slightly increased in *Ficus* and *Jacaranda*. Chaturvedi *et al.*, 2013 reported that RWC was considerably reduced in polluted sites, and significantly negatively correlated with dust (Rai and Panda, 2013). Higher RWC in plants will help maintain their physiological balance when exposed to air pollution and favors drought resistance. In the present study, *Ficus religiosa* and *Jacaranda mimosifolia* showed increase in mean RWC value at polluted sites, indicating their better tolerance than others.

Specific leaf area (SLA)

In all studied tree species specific leaf area (SLA) decreased at polluted sites than control sites and similar results were also reported by Abbasi *et al.*, 2018) in Tehran Metropolitan City, Iran. Reduction in SLA reduces water loss and transpiration. This is possibly a strategy of plants to improve stress tolerance (Xu *et al.*, 2009). Plants that are resistant to tension have higher relative water content (Mahecha *et al.*, 2013), which is evident in *Ficus religiosa* and *Jacaranda mimosifolia* in the present study. Insignificant relation was mostly observed between the % decrease in SLA and biochemical parameters, which might be due to various strategies and physiological adjustments in plants to survive in adverse conditions.

Significant negative correlation was observed between % decreased in SLA and Zn accumulation in *Populus*, *Jacaranda*, *Callistemon* and *Cinnamomum* growing at polluted sites. In the present study the Zn accumulation in these plants are below the phytotoxic level as suggested by Chaney, (1993) but even then reduction % in SLA ranged from 9 to 50% in *Populus*, 13 to 73% in *Jacaranda*, 21 to 78% in *Callistemon* and 10 to 32% in *Cinnamomum*. This reduction in SLA might be due to deficiency of some other essential elements when there is stress in plant due to increased concentrations of one toxic metal (Sharma and Chettri, 2005). Significant positive relation was observed between the % decreased in SLA and Cu accumulation in *Ficus* and *Cinnamomum* and found in low concentrations of Cu in both these species. Cu is essential for various physiological processes such as a structural element in regulatory proteins, electron transport, mitochondrial respiration and oxidative stress responses (Marschner, 1999; Raven *et al.*, 1999) in low concentrations. The % decrease in SLA ranged from 25-53% in *Ficus* and 10-32% in *Cinnamomum.* The positive relation between them might be due to some other factors, which regulates SLA, but not the toxicity of Cu. Significant negative correlation was observed between Cu accumulation and % reduction of SLA in *Jacaranda*. In the present study, the highest accumulation of Cu was found in *Jacaranda mimosifolia* and similar results were also reported by Zeynep *et al*., (2012). The negative relation between them might be due to the concentration of Cu which reached up to 20.60 mg/kg at polluted sites and are at the phytotoxic range (Padmavathiamma and Li, 2007). The significant positive relation between % reduction of SLA and Pb content might be due to Pb toxicity. Markert (1994) reported the phytotoxic range of Pb for plants to be between 3 and 20 mg/ kg. As the Pb concentrations at the polluted sites reached up to 27.51 mg/kg in *Populus*, 39.61 mg/kg in *Ficus*, 37.8 mg/kg in *Grevillea* in the present study, and these concentrations are at phytotoxic range in plants as described by Markert (1994).

Conclusion

From the above it can be concluded that though the studied biochemical parameters have their physiological role in leaves but their relation with SLA is mostly insignificant which might be due to various strategies for physiological adjustment in stressed conditions in plants. The impact of heavy metals in small concentrations on SLA are not evident, but when present in high concentrations i.e at phytotoxic range, their impacts on SLA is conspicuous. Based on heavy metal accumulation and SLA, all the studied roadside trees (like *Populus euroamericana, Ficus religiosa, Jacaranda mimosifolia, Grevillea robusta, Callistemon lanceolatus* and *Cinnamomum camphora*) which could accumulate Pb and/or Zn at high concentrations at polluted sites and could withstand adverse environment by reducing their SLA are recommended for future plantation to mitigate roadside air pollution problem.

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